Introduction

The regulatory guidelines recommend that in a human AME study, metabolites formed at 10% of the total drug-related exposure should be characterised. Therefore early evaluation of a drug’s metabolism is important to make informed decisions regarding the most appropriate human radioactive dose.

Prior to initiation of the protocol for the radiolabelled clinical AME study, metabolite profiling data from the analysis of non-radiolabelled plasma samples taken from both preclinical and first-in-human (FIH) pharmacokinetic (PK) studies were analysed, and the data used to support the choice of a dose level suitable to meet these regulatory guidelines.

Methods

Plasma samples from preclinical species and human subjects were prepared by protein precipitation with acetonitrile. Extracts were concentrated by evaporation, reconstituted in mobile phase and submitted for analysis using a high-resolution ThermoFisher LTQ Orbitrap® hybrid mass spectrometer connected to a ThermoFisher Accolade® UPLC system via an API interface. Data were interrogated for the presence of metabolites based on the accurate masses of potential metabolites. Peak areas of parent compound and detected metabolites were determined in the appropriate extracted ion chromatograms (EIC). From these values the relative exposure of drug and its major metabolites were compared across the species. The most abundant potential metabolites were targeted for characterisation by LC-MS/MS.

Non-Radiolabelled Species Comparison Study

Plasma samples from toxicity studies (rat, mouse and dog) together with human plasma samples from a Phase I, single and multiple oral dose pharmacokinetic and pharmacodynamic study, were initially analysed to assess the potential for unique or disproportionate human metabolites formation; with the results used for safety assessment. As the regulatory guidance suggests that metabolite exposure in human should be assessed at steady state, the number and identities of metabolites were determined in plasma samples taken following repeated administration to each species. Matrix matching experiments were performed to correct the peak area response data for matrix suppression differences across species.

Non-Radiolabelled Human Metabolite Profiling—Subject and Dose Level Variability

The metabolite profiles of additional human plasma samples taken from a non-radiolabelled Phase I PK study were characterised to gain information regarding variability in metabolism between individual subjects (Figure 3), and between different dosing regimens (Figure 4). The following relative metabolite exposure data were generated.

Following repeated administration of the test drug, the two most abundant human metabolites consistently accounted for approximately 10% of the total drug related exposure, with little variation between subjects. Across the dose levels, no notable differences in exposure were observed, indicating no significant accumulation of the drug or its metabolites with increasing dose.

[14C] Radiolabelled Human AME Study

Although not quantitative, the data from the non-radiolabelled studies had indicated the potential for disproportionate human metabolites to be present at a level which may be of concern from a regulatory perspective. Consequently, in advance of the radiolabelled clinical AME study, predictions of the likely concentrations of radioactivity in the human plasma samples were made to ensure that these metabolites, which potentially could be formed at 10% of the total drug-related exposure or less, could be adequately quantified by radio-HPLC.

These calculations were based on the following factors:

- the non-radiolabelled parent compound plasma concentration data
- the specific activity of the proposed clinical dose formulation
- the number and proportions of metabolites as indicated from the non-radiolabelled metabolite profiles
- sample preparation and concentration techniques

Based on these predictions (Table 1), the proposed clinical dose was increased from 0.5 mCi (100 µCi) to 1 mCi (200 µCi) to ensure a suitable chromatographic response.

Plasma samples were analysed by radio-HPLC, using fraction collection with offline radio-detection (Figure 5). The responses (dpm) of peaks formed at 10% of the total peak area were close to the predicted values (Table 2). Peaks could be quantified over sufficient time-points to determine relative exposure (AUC) data for the most abundant metabolites.

The increased radioactive dose level ensured that there were sufficient radioactivity in the resulting human plasma samples to quantify and identity metabolites formed at less than 2% of the total drug-related exposure without the need to move to other techniques (i.e. AME). The quantitative radio-HPLC profiling data also confirmed that the potentially disproportionate human metabolites were below the exposure level which would have been of regulatory concern.

Conclusions

High-resolution mass spectrometry was successful in characterising the most abundant plasma metabolites in samples taken from FIH studies. The data obtained were used to predict the chromatographic response of the drug and its metabolites in the plasma profiles from the radiolabelled human study. Therefore, understanding the metabolism of a compound at an early stage in its development can be used to ensure that the objectives of the radiolabelled clinical AME study can be met, and the regulatory requirements satisfied.

Reference